Evaluation of the Interference of HbE on HbA1c Results on the Detection System Produced by Shenzhen Lifotronic

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Abstract

Objective: To analyze the interference of HbE on the HbA1c results on detection systems manufactured by Shenzhen Lifotronic.

Methods: Volunteers were recruited from Zhongshan city, Guangdong province during June 2020 to November 2020. A total of 55 specimens were obtained, including 40 cases without HbE(23 cases in normal group and 17 cases in diabetes group) and 15 cases with HbE variant. HbA1c of these two groups (normal and HbE group) were analyzed by Shenzhen Lifotronic H8, H9 and GH-900Plus, Bio-Rad Variant II Turbo 2.0 and D-10 from USA, Arkray HA-8180 from Japan, statistical analysis was conducted on the difference between detection results of normal group and HbE variant group. Bio-Rad Variant II Turbo 2.0 was used as the comparison system to calculate the bias and to evaluate the interference of HbE on each system.

Results: In normal group, the results of each detection system were comparable to those of Variant II Turbo 2.0, with a bias of -6%~6%. In HbE variant group,system bias of H8, H9, GH-900Plus and D-10 was within -6% and 6%, and the results showed no significant difference ($P>0.05$), while the negative bias of HA-8180 showed significant difference ($P<0.05$).

Conclusion Compared with Bio-Rad Variant II Turbo 2.0, HbA1c can be correctly detected by H8, H9 and GH-900Plus system without the interference of HbE.

Introduction

Hemoglobin A1c (Glycated hemoglobin, HbA1c) is widely used in clinical practice as an important indicator to assess the average level of blood glucose control. China has paid increasingly attention to the standardization of HbA1c test results, and the research and development of HbA1c test system has been constantly innovating. It is particularly important to develop a detection system with good precision and strong anti-interference ability to accurately meet the needs of clinical detection [1,2].HbE is one of the most common abnormal hemoglobin proteins in the world, with high incidence rate in China, India, Southeast Asia and North America [3]. Hemoglobin variation can interfere with HbA1c test results to varying degrees [4], the purpose of this study is to discuss the HbA1c testing results in the presence of HbE variant by Shenzhen Lifotronic's H8, H9 and GH-900Plus detection systems. The comparison between Shenzhen Lifotronic's three system and Bio-Rad Variant II Turbo 2.0 with regard to the interference degree of HbE was evaluated. The results are reported as follows.

Materials And Methods

Source of specimens

Volunteers from Zhongshan Hospital of Sun Yat-Sen University were recruited from June 2020 to November 2020. A total of 55 EDTA-K_2 anticoagulant venous blood samples were obtained in this study, including 40 cases in normal group,which were used as normal specimen for comparison experiment without hemoglobin variant, hyperlipidemia, uremia and other interfering factors. In addition, 15 HbE variant samples were preliminarily determined HbE presence by capillary electrophoresis, and then genotype was detected and confirmed by gene sequencing. All the volunteers signed informed consent forms, and the study was reviewed by the Medical Ethics Committee of Zhongshan Hospital.

Specimen collection

Fasting blood of 2mL EDTA-K_2 anticoagulant venous blood was collected. These samples were used for capillary electrophoresis, gene sequencing and HbA1c test. All collected specimens were pipetted and stored at -70℃.

Detection system and method

HbA1c was detected by Ion Exchange High Performance Liquid Chromatography (IE-HPLC) using Shenzhen Lifotronic's automatic H8, H9 and GH-900Plus HbA1c analyzers and reagents, calibrators, control material and consumables.

HbA1c was detected by Ion Exchange High Performance Liquid Chromatography using Variant II Turbo 2.0, D-10 automatic HbA1c analyzers and reagents, calibrators, control material and consumables (Bio-Rad Company).

HbA1c was detected by Ion Exchange High Performance Liquid Chromatography using Arkray HA-8180 automatic HbA1c analyzer and reagents, calibrators, control material and consumable.

Sample Detection

55 specimens were tested by H8, H9, GH-900Plus, Variant II Turbo 2.0, D-10, and HA-8180 respectively. The NGSP quality evaluation material was retested at Variant II Turbo 2.0 before experimental operation to ensure that the system met the requirements of NGSP laboratory certification, so as to ensure the traceability of the comparison system. The relative bias between detection results of different systems and Variant II Turbo 2.0 was calculated to be within -6% ~ +6%. Meeting this condition means that detection results of other systems are comparable to those of Variant II Turbo 2.0.

Statistical analysis

HbA1c tests were respectively performed on the experimental systems and the comparison system for each group. Statistical analysis was carried out for the bias of detection results. All the data were statistically analyzed by Excel and SPSS 22.0 software. Kolmogorov-Smirnov test, a single sample, was used to test the normality of the measurement data, which were in line with the normal distribution, and paired sample t-test was used as well. $P<0.05$ was considered statistically significant.

Results

Retesting results of NGSP in Variant II Turbo 2.0 system

All the NGSP quality assessment and target value bias detected on the same day were less than 6%, detailed results are shown in Table 1. Indicating that the accuracy of the analysis and testing system reaches acceptable standards to ensure that the system meets the requirements of NGSP laboratory certification, so as to ensure the traceability of the comparison system.

Table 1
Retesting results of NGSP quality evaluation in Variant II Turbo 2.0 system

Serial No.	NGSP target value	Value 1	Bias %	Value 2	Bias %	Value 3	Bias %
1	5.54	5.6	1.08	5.6	1.08	5.5	- 0.72
2	6.31	6.2	- 1.74	6.3	- 0.16	6.3	- 0.16
3	9.64	9.5	- 1.45	9.6	- 0.41	9.6	- 0.41

Comparison and bias analysis of HbA1c test results of the five detection systems

The correlation coefficient r of regression analysis between the five detection systems including H9, H8, GH-900Plus, D-10 and HA-8180 and the comparison system of Variant II Turbo 2.0 were all greater than 0.975, and the percentage bias of the experimental system and the comparison system were all less than ±6%, the specific results were shown in Table 2.

Table 2
Comparison of HbA1c results between the five detection systems and comparison system

The experimental system	The regression equation	The correlation coefficient	Normal group bias range %
H9	Y = 0.967x + 0.235	0.997	- 2.9 ~ 5.7
H8	Y = 0.961x + 0.293	0.997	- 2.6 ~ 3.8
GH-900Plus	Y = 0.967x + 0.235	0.998	- 1.7 ~ 5.9
D-10	Y = 0.966x + 0.184	0.997	- 5.2 ~ 3.5
HA-8180	Y = 0.957x + 0.307	0.995	- 3.8 ~ 5.7

Chromatogram analysis of HbE variant in different HbA1c detection systems

The elution time of H9 was 360 seconds, and the peak names were HbA1a, HbA1b, HbF, LA1c, HbA1c, P3, P4, HbA0 ,HbA2 and HbE/D/S/C (if any) respectively. The average retention time of HbE was 220 seconds, which is after HbA2. Moreover, it was identified and reported separately in the chromatogram, and the system could directly indicate suspected HbE variant.

The elution time of H8 and GH-900Plus was 130 seconds, and the peak names were HbA1a, HbA1b, HbF, LA1c, HbA1c, HbA0 and variant window respectively. The average retention time of HbE was 89 seconds which is after HbA0 in the chromatogram, and the system gave V-window alarm.

The elution time of Variant II Turbo 2.0 was 90 seconds, and the peak names were HbA1a, HbA1b, HbF, LA1c, HbA1c, P3, P4 and HbA0 respectively. The average retention time of the variant was 66 seconds, HbE's retention time is after HbA0, and it was next to HbA0, but the obvious presence of this variant could be identified from the chromatogram, and the system gave V-window alarm too.

The elution time of D-10 was 180 seconds, and the peak names were approximately HbA1a, HbA1b, HbF, LA1c, HbA1c, P3, and HbA0, respectively. The average retention time of HbE variant was 95 seconds, which is after HbA0, and it was next to HbA0, but the obvious presence of this variant could be identified from the chromatogram, and the system gave V-window alarm too.

The elution time of HA-8180 was 48 seconds, and the peak names were approximately HbF, LA1c, HbA1c, and HbA0, respectively. The retention time of HbE was between HbA1c and HbA0, and it was after HbA1c in the chromatogram. The system showed no variant alarm or there was no peak name or retention time.

Statistical analysis of HbA1c test results differences between normal group and HbE variant group.

There was no significant difference between HbA1c and HbE group detected by H8, H9, GH-900plus, Turbo 2.0 and D-10 ($P>0.05$), while there was significant difference between HbA1c and HbE group detected by HA-8180 ($P<0.05$), as shown in Table 3.

Table 3
HbA1c test results of normal group and HbE group (%)

The experimental system	normal group HbA1c %	HbE variant group HbA1c%
H9	5.69 + / - 0.38	5.48 + / - 0.52
H8	5.66 + / - 0.43	5.55 + / - 0.55
GH-900Plus	5.75 + / - 0.41	5.65 + / - 0.56
Turbo 2.0	5.60 + / - 0.42	5.50 + / - 0.53
D-10	5.60 + / - 0.42	5.48 + / - 0.47
HA-8180	5.65 + / - 0.45	4.44 + / - 0.28▲
Note: ▲Comparison of HbA1c results between normal group and HbE group (P<0.05)		

Bias analysis of HbE variants in different HbA1c detection systems

D-10, H9, H8 and GH-900plus showed no significant difference (P>0.05) with the compared system(bias <±6%), while HA-8180 showed significant difference (P<0.05) with negative bias (P<0.05). As shown in Table 4 and figure 2 for details.

Table 4
Average bias of each experimental system and comparison system (%)

The experimental system	Average bias % of HbE-free group	Average bias % of HbE group
H9	0.45 + / - 2.42	0.34 + / - 1.99
H8	0.91 + / - 1.63	0.94 + / - 1.58
GH-900Plus	2.34 + / - 2.07	2.66 + / - 2.44
D-10	0.47 + / - 2.05	0.25 + / - 1.59
HA-8180	0.52 + / - 2.88	18.95 + / - 4.25▲
Note: ▲ Comparison of HbA1c results with comparison of system bias in HbE-free group and HbE variant group, P<0.05		

Discussion

Methods for the detection of HbA1c include enzymatic method, immunoturbidimetry, affinity chromatography and ion-exchange liquid chromatography, among which the results of ion-exchange

liquid chromatography (IE-HPLC) are more accurate with good repeatability and reproducibility. American Association of Clinical Chemistry (AACC) and International Federation of Clinical Chemistry (IFCC) suggest to use IE-HPLC as the one of the gold standards for the detection of HbA1c. Therefore, HPLC method is widely used in clinical practice. However, different detection systems based on HPLC method will report different HbA1c results with presence of variant due to the differences in column quality, reagent formulation and elution time, etc. [5].

Professor Wen Dongmei and Xu Anping's research results show [6,7] that the Variant II Turbo 2.0 has long testing time with high resolution. In addition, the elution time was 90 seconds, thus HbE variant peak can be well separated. Therefore, the system is not intefered by HbE variants and the results are consistent with NGSP web site. Moreover, testing lab for this experiment was accredited by NGSP as grade I laboratory, results accuracy is guaranteed and acceptable by different requirement. Therefore, Variant II Turbo 2.0 was chosen as the comparison of the experimental system to evaluate the HbE anti-interference ability.

These three HbA1c detection systems from Shenzhen Lifotronic have anti-interference ability to HbE variants. This study also shows that none of the HbE variants have any influence on the results of these three detection systems. H9 system variant mode was 360 seconds(graph is 300 seconds only) and H8/GH-900Plus is 130 seconds. The chromatograms showed that HbE could be recognized by the detection systems and could be well separated. Among all the detection system used in this experiment, H9, H8, GH-900Plus and Variant II Turbo 2.0 system had the same degree of HbE variant recognition, indicating the presence in the Variant-Window.H9 variant mode can fully separate HbE variant, and the retention time of HbE is around 220s, which is about 0.73 quantile of the total graphic time.This parameter for H8 is 0.68, GH-900Plus is 0.68, and Variant II Turbo 2.0 is 0.73. Retention time of HbE in these three Lifotronic systems is highly similar to Variant II Turbo 2.0.Other studies have shown that [8] the H9 system, although has a relatively low resolution of Hb NewYork, could indicate the presence of the variant through the chromatogram. The variant mode of H9 could identify 12 hemoglobin variants and preliminarily determine the type of the variant based on the retention time and peak shape of the variant. Due to relatively large number tests that can be supported by chromatographic column and reagent of H9 system, it reduces the frequency for replacement of chromatographic column and making calibration/QC in laboratory.It also helps the lab to save costs and ensures the stability of test results, so it is more suitable for large laboratories.

Compared with Variant II Turbo 2.0, D-10 system manufactured by Bio-Rad from USA has a longer elution time of 180 seconds, and the HbE Variant can be completely separated. Therefore, the anti-interference ability of HbE is consistent with that of Variant II Turbo 2.0 manufactured by the same company. However, its elution time is longer, and the number of tests can be supported by chromatographic column and reagent is less, so D-10 is more commonly used in small laboratories.

The Arkray HA-8180 from Japan has the feature of fast speed.. Its elution time is 48 seconds, which is the fastest in the detection systems of this study. Its fast mode is often used for mass screening.

However, the elution time is too short to separate the HbE peak which leads to relatively large negative bias in the detection results. Therefore, HA-8180 was not suitable for the detection of HbA1c results for population with HbE variants.

In conclusion, H8, H9 and GH-900Plus HbA1c detection systems and Bio-Rad Variant II Turbo 2.0 have comparable results when testing HbA1c in population with HbE variants. And the anti-interference ability of these three detection systems from Shenzhen Lifotronic was better than that of Arkray's HA-8180. With continuous improvement of reagent formula and chromatographic column quality, HbA1c detection systems from Shenzhen Lifotronic have strengthened their ability to separate and identify the variants and have a good performance on anti-interference of variants. In regions where hemoglobin variants appear frequently, it is necessary to fully understand the anti-interference ability of each detection system against the variants, so as to avoid results bias of HbA1c, which would lead to misjudgment of average blood glucose when monitoring level of patients by clinicians.

Declarations

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Thanks to all the volunteers from Zhongshan.

Ethical approval:

Informed consent was obtained from all individual participants.This study protocol conforms to ethical guidelines of World Medical Association. The Medical Ethics Committee of Zhongshan Hospital reviewed this protocol, and all volunteers gave their informed consent for the inclusion in this study.

Approval of the research protocol:

This study was approved by Zhongshan Science and Technology Bureau.

Informed Consent:

Informed consent was obtained from all individual participants included in the study.

Approval date of Registry and the Registration No: Zhongshan Science and Technology Bureau approved this study on January 2018.(Registration No. 2018B1036)

Animal Studies:

N/A

Conflict of interest:

The authors declare no conflict of interest.

Data Policy:Some or all data, models, or code generated or used during the study are available from the corresponding author by request.

Authors' Contributions:

Hu YZ and Suo MH are responsible for the design of experimental scheme, the collection of samples and the writing of articles.

Hu YZ and Suo MH contributed equally to this work.

Xu Y and Hu WY are responsible for the arrangement of instrument detection principle, time and advantages.

Hu T and Huang JM are responsible for searching the experimental population, contacting the volunteers by telephone, and guiding the volunteers to collect samples. Wang WJ and Chen K responsible for the collection, detection and preservation of specimens.

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Figures

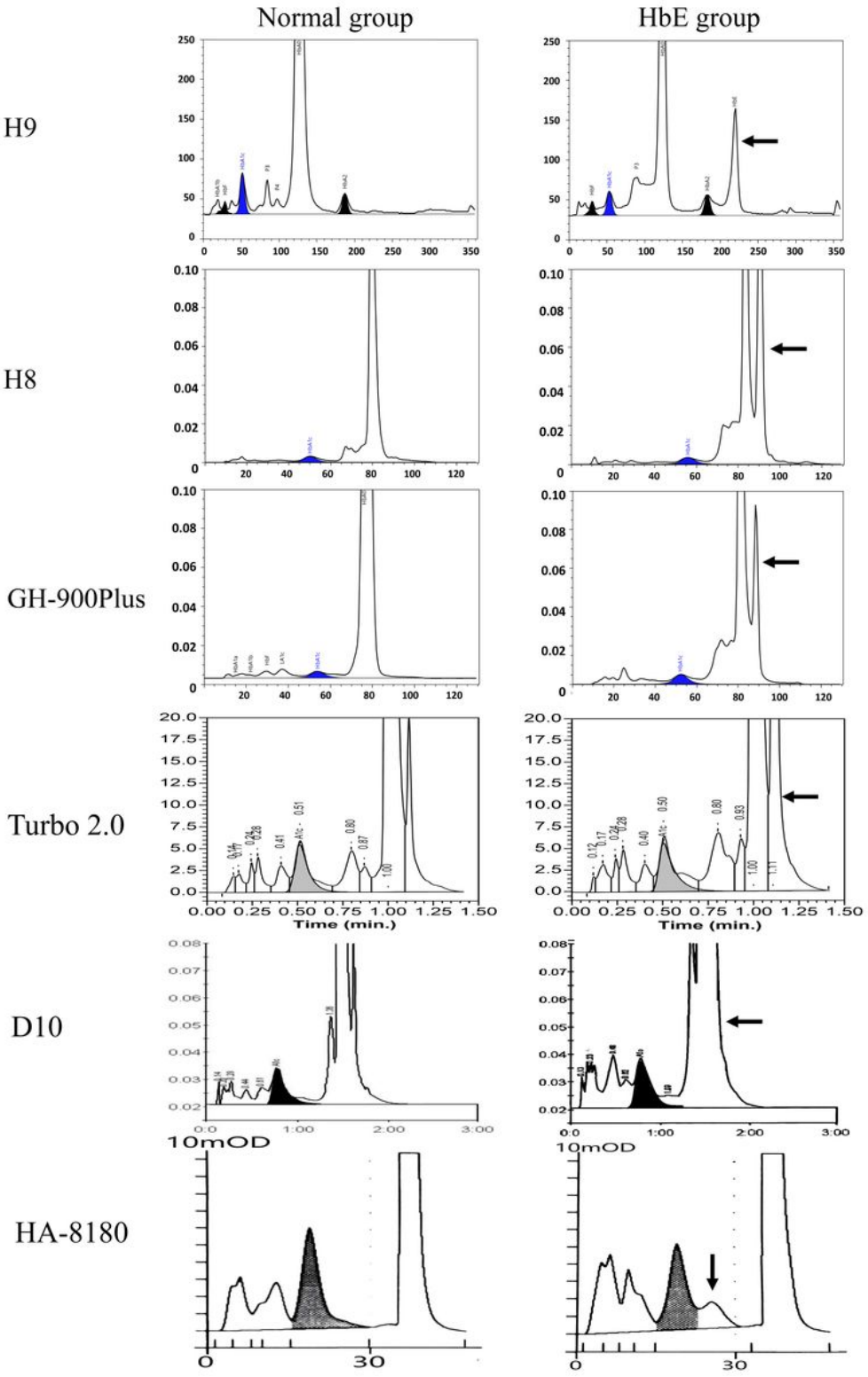

Figure 1

Chromatograms of normal and HbE group in 6 detection systems ← - Represents the HbE position in Chromatogram

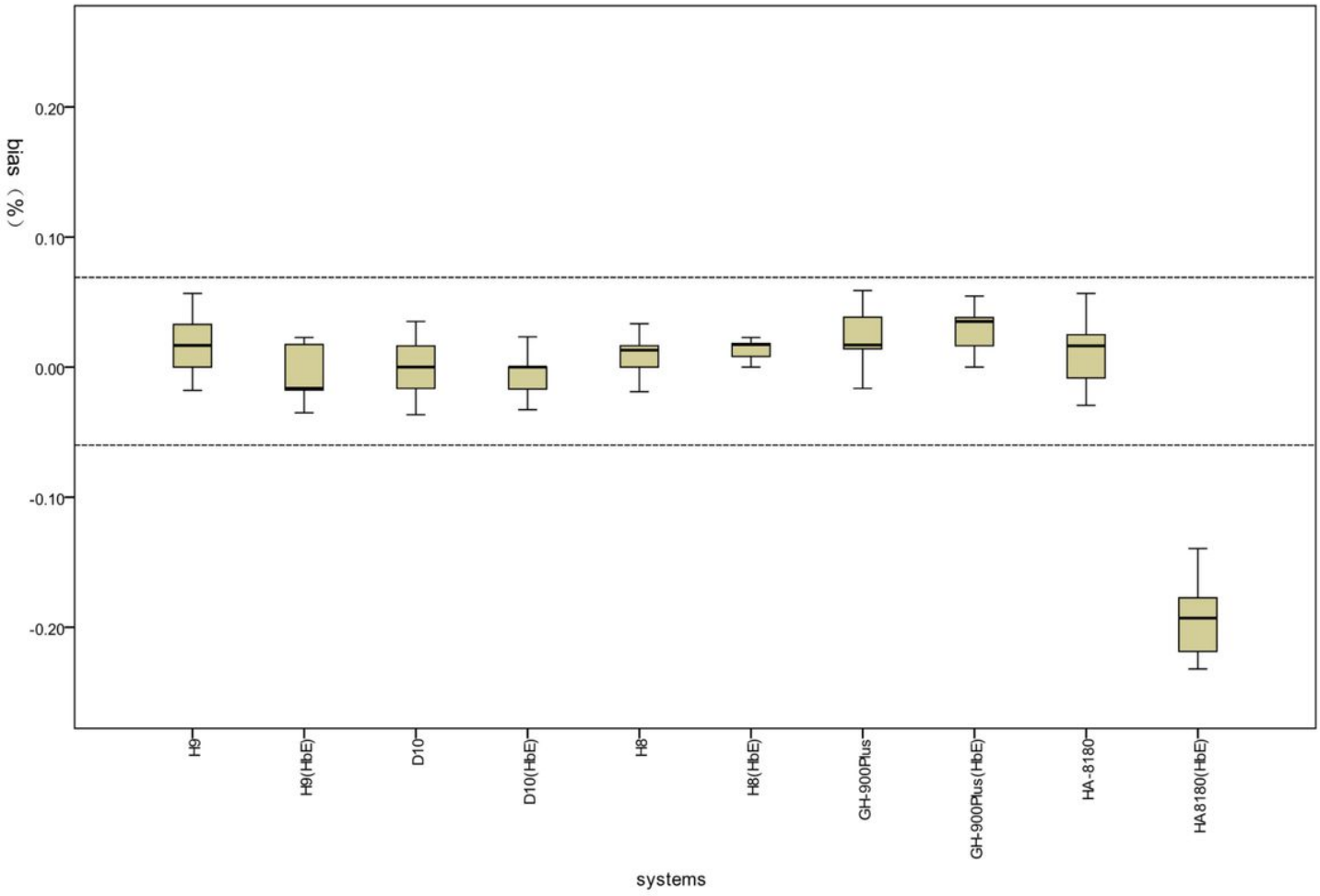

Figure 2

5 Bias distribution of HbA1c detection results in 5 detection systems X-axisrepresents different systems Y-axis represents bias between test results and comparativemethod(%) Dottedlines representbias within -6% ~ +6%.